IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

SERIAL NO.:	256,689	§	DOCKET D-5050
		§	
FILING DATE:	October 12, 1988	§	GR ART UNIT: 187
		§	
APPLICANT:	CHARLES T. CASKEY, ET AL	§	EXAMINER:
		§	A. Marschel.
TITLE:	MULTIPLEX GENOMIC DNA § AMPLIFICATION FOR DELETION DETECTION	§	
		§	

DECLARATION UNDER 35 C.F.R. 1.132

Dear Sir:

I, Richard A. L. Gibbs, do hereby depose and say as follows:

1. I have read U.S. Application Serial No. 256,689 filed October 12, 1988. I am aware of the contents of said application.

2. I am an assistant professor of molecular genetics in the Institute for Molecular Genetics at Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030. I am skilled in the area of molecular biology, oligonucleotide probes and synthesis of oligonucleotide probes and binding of oligonucleotide probes to DNA. A resume describing my experience is attached to this declaration.

3. I have personally been involved in the investigations described in the above-identified application and in this affidavit.

4. Experiments using the multiplex procedure for hypoxanthine-guanine phosphoribosyl transferase are described in the attached reference, R. A. Gibbs, et al, Genomics 7:234-244 (1990). The reaction conditions in the paper are essentially those shown in the specifications of the above-referenced application. The actual primers used are shown in Figure 1.

5. Conclusion. As can be seen from the attached reference, multiplex analysis works for gene loci other than the gene loci for Duchenne muscular dystrophy. Further I have seen and discussed the results of Dr. Grompe and Dr. Ballabia for hexosaminidase A, glucocerebrosidase, steroid sulfatase, β globin,

Amplification reactions were performed in a Perkin-Elmer/Cetus thermocycler using the step cycle functions. The reaction was controlled by regulated and repetitive temperature changes of various duration. The reaction was heated initially to 94°C for seven minutes. Subsequently about 30 cycles of the following temperature durations were applied 94°C for one minute and 55°C for 45 seconds and 65°C for 3-1/2 minutes. Following completion of the final cycle the reaction was incubated at 65°C for an additional seven minutes. Reactions were then stored at -4°C until analysis.

b. The Probes Used. Primers to the following loci were used: hexosaminidase A, glucocerebrosidase, β globin, α-1-antitrypsin and cystic fibrosis locus, ΔF_{508} loci were used.

c. A typical result of this amplification of multiplex analysis is shown in the attached figure.

5. Conclusion. As can be seen from the attached figure, multiplex analysis as performed in the above-described procedure and as described in the above-referenced patent application works for gene loci other than the gene loci for Duchenne muscular dystrophy.

I hereby declare that all statements made herein on my own knowledge, are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code.

Date: 4-10-91

M. Grompe, M.D.

Marcus Grompe, M.D.